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THE RELATION OF THE EMBRYO TO THE PRINCIPAL AXIS OF SYMMETRY IN THE BIRD'S EGG.

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While studying the earliest stages in the development of the pigeon's egg some years ago it became necessary to devote some attention to the laid egg. Here I found several relations of fundamental importance which recent embryologists have consistently neglected. It was especially interesting to find that the well-known relation between embryo and egg as a whole (Fig. 1) is subject to much greater variation than is generally believed. This became still more striking when it was found that the eggs of a single bird vary less than the eggs obtained from a group of birds. The present report covers observations extended over a much longer period than my former one (1912), together with an analysis of the data and the literature.

I wish to call particular attention to the older studies on the bird's egg. From the time of Aristotle until the work of Kölliker, O. Hertwig and van Beneden began, the only accredited and accessible embryological material was afforded by the hen's egg and a wealth of observation was accumulated concerning it. Yet recent students of the bird's egg have dated their work from Gegenbaur's paper in 1861 assuming that the monumental researches of Harvey, Malpighi, Wolff, Dutrochet, Coste and even Purkinje and von Baer have now only an historic interest. The folly of this will appear from what follows. These workers, unhampered by our elaborate technical methods, studied this macroscopic egg with the thoroughness of studious leisure and then wrote of it in a manner that must arouse the wonder and admiration of any embryologist today. The neglect which Purkinje has suffered can be explained at least in part, but the oblivion in which v. Baer's work still languishes is incomprehensible.

The findings reported here are significant not alone in illuminating the nature and causes of the variability in the relations of the axes of bilaterality. They have also a direct bearing upon all descriptive studies of the earliest stages of the bird's egg and they must especially be taken into account in the interpretation of experimental studies of stages preceding the appearance of the primitive streak.

In order to discuss the literature it will be necessary to begin with a general statement of our knowledge concerning the axes of the bird's egg, working backwards from the incubated egg to the ovarian oöcyte.

After the appearance of the primitive streak the bird's egg presents two obvious axes of symmetry; one a general axis of the egg as a whole, the principal axis, the other the axis of the embryo itself. Both of these are more or less apparent in other eggs, especially the highly meroblastic eggs of reptiles and selachians, but so far as is known it is only in birds that these two axes are definitely related to one another. The fact that they *are* related indicates that they are expressions of the same (bilateral) symmetry of the organism—a thesis for which I brought forth abundant evidence in 1912. Unfortunately we

have few data as to the relation of the two axes in birds other than the hen and pigeon but such observations as we do have indicate that many if not all birds' eggs show the same general relations. Duval in 1884 assumed this to be true but he never published any evidence.

The principal egg axis is defined by the form of the secondary egg envelopes. In addition to the obvious difference between the two ends of the shell, the ends of this axis are differentiated by even more constant characters like the position of the air space, pigment distribution in certain forms, and less dependable

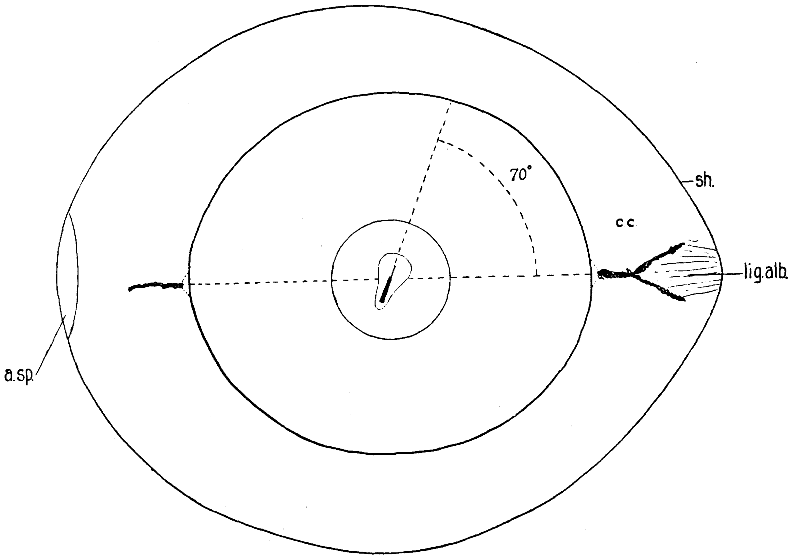


FIG. 1. A diagrammatic polar view of an entire pigeon's egg showing the relation between the embryonic axis and the various features of the principal egg axis. The right side of the embryo is nearer the end of the egg which passed *first* down the oviduct, *i. e.*, the pointed end of the shell. In the "uterus" the pointed end of the shell is directed toward the cloaca, the blunt end with the air space toward the infundubulum of the oviduct. The principal egg axis, (———) is marked by the major axis of the shell, the air space (*a.sp.*), the ligamentum albuninis (*lig.alb.*), the chalazal axis (*c.c.*, cloacal chalaza), and the long axis of the ovum.

characters such as the size differences between the chalazæ. All of them are expressions of the structure and activity of the oviduct and their relations are constant because the egg is definitely oriented in the oviduct. As this point of view is new

it is necessary to call to mind these characters and their relations to one another.

The two ends of the principal egg axis are differentiated by the following characters:

1. The relation of the embryo to the principal axis is such that its right side is nearer one end of this axis, its left side nearer the other.

2. *Shape of Shell.*—The two ends of the shell differ from one another in the eggs of most birds so that we may usually speak of a blunt and a pointed end.

3. *Pigment.*—The eggs of many birds, particularly among the Passeres are more or less spotted with pigment. The characteristic flecks, blotches, streaks or lines are often unequally distributed over the shell so as to differentiate between the two ends of the principal axis. Sometimes there is a pigment wreath around the blunt end as in many warblers, sometimes the spots are irregularly scattered over the blunt end as in certain fly—catchers and in still others there are spots over most parts of the shell but they are more abundant over the blunt end (certain warblers), and finally in some heavily pigmented eggs like those of the English sparrow the pigment is scantier at the pointed end than elsewhere. The reason for this is obvious when one considers the mode of origin of this pigment and the orientation of the egg in the oviduct (cf. p. 333). This character, then, is a well-defined differentiation of the long axis.

4. *Air Space.*—In the laid egg the air space is always at or near the blunter end of the shell. This is an exceptionally constant character.

5. *Chalazæ.*—The chalazæ are normally attached to the yolk in the line of the principal axis so that the chalazal axis is merely another expression of this axis. Furthermore, one often finds that one chalaza is larger and more distinct than the other. It is almost invariably the one at the pointed end of the shell. During incubation the blunt end chalaza is more likely to become detached or to disappear entirely; that is to say it reverts more readily to the sol phase.

6. *Ligamentum Albuminis.*—The second or thicker albumen is more or less firmly attached to the inner shell membrane,

usually at both ends of the shell but this prolongation through the outer thin albumen is more marked at the pointed end in the laid egg. This prolongation Tredern fancifully called a "ligament."

7. *Long Axis of Yolk.*—The striking fact that the yolk (ovum) is not a sphere has been generally overlooked. One of its axes is greater than either of the other two and this axis is perpendicular to the polar axis and coincides with the principal axis.

8. *The Oviducal Orientation.*—All of our evidence points unmistakably to the conclusion that the pointed end of the egg passes *first* down the oviduct. We may therefore speak of a cloacal and an infundibular end of the egg. The principal axis therefore, coincides with the oviducal axis and the differences which we find between the two ends of the former are due to differences in the activity of the oviduct. The long axis can be identified in all ovarian oöcytes and at the time of ovulation the ovum is oriented in the oviduct with reference to its long axis. The long axis accordingly determines the principal axis as we see it in the laid egg. These relations are of fundamental importance for they demonstrate that the embryo is definitely related to an ovarian axis of symmetry.

9. I was able to show that the end of the long axis which is to pass first down the oviduct is predetermined in the ovary by the position of the latebra of Purkinje.

10. Finally the eccentric position of the latebra is determined by the corresponding position of the germinal vesicle in young oöcytes.

In view of these facts we may express the relation between egg and embryo thus: *The right side of the embryo is nearer that end of the principal egg axis which is predetermined in the ovary to pass first down the oviduct.* This holds of course only for stages previous to the turning of the embryo on its side. To state the case in the more usual way we should say: If the end of egg which is predetermined to pass first down the oviduct be held to the observer's right the head of the embryo will be directed away from him. The presence of an axis of symmetry in the ovarian oöcyte so definitely related to the embryonic axis naturally led me to look for morphological evidences of the embryonic axis

itself. The evidences I found in ovarian and oviducal eggs were enumerated in my 1912 paper and I hope soon to publish the details of these observations.

II. Historical Review.

A. *The Principal Axis of the Egg.*—Aristotle devoted much attention to the hen's egg and he speculated at length upon the difference between the two ends of the shell. The Aristotelian doctrine, which is to be found elaborated even in some relatively recent papers may be stated as follows: The ovum drops into the oviduct as an apple from the tree; the ovarian pedicle of the oöcyte becomes the pointed end chalaza which passes *last* down the duct and the theca folliculi becomes the shell membrane. The pointed end is the "principium ovi, where the first rudiments of the egg are fashioned." This philosophy was based chiefly upon two observations of Aristotle's: The ovarian stalk or pedicle is characteristically long in the hen's oöcyte and the blunt end of the egg is laid first. Harvey's account of the early development is the first clear and accurate one for he understood the nature and destiny of the "cicatricula." He was able therefore to correct the error concerning the "principium ovi" as well as many others which his predecessors had made. However much he chuckled over the blunders of others, Harvey was always at some pains to explain or extenuate the errors of the Great Master. His discussion of Aristotle's statement that the shell does not harden until it reaches the outer air is well worth reading (1651, Exercitatio XI.).

B. *The Air Space.*—Harvey was the first to observe the air space carefully; he found it at or near the blunt end of the hen's egg and he cites a common belief that if it is at the center of the blunt pole a male will be produced. He describes it as very small at laying, growing larger as incubation advances. v. Baer (1828) was the first to note that it is not present at all in oviducal eggs nor at the moment of laying. Coste (1847, p. 306) is the only one apparently, who has seriously considered the problem of the air space. He confirmed v. Baer's observations and showed that as the egg cools after laying atmospheric air enters through the shell and forms the space between the two layers

of the shell membrane. He did this by killing a hen that was about to lay, ligating the uterus at either end and immersing the whole in a dish of oil. The uterus was then removed and the next day an oil-filled cavity was found in the place of the air space. But why should this space always arise at the blunt end? Coste was able to produce it under any part of the shell by a modification of his procedure. If, instead of removing the uterus under oil he clipped a window in it (thus exposing any given area of the shell) he obtained a typical oil-filled cavity under the window between the shell membranes. He was not able to explain why it normally forms where it does. Valentin (1835, p. 30) had previously attributed the formation of the air space to the puckering of the oviducal walls at the junction of "isthmus" and "uterus." He cites Purkinje to this effect but I have not found any mention of the matter in either edition of his paper. The region of the shell where the space forms is obviously more porous, quite possibly for the reason which Valentin gives. I have made some preliminary tests and am inclined to think that the shell cuticle is concerned in this localization.

It would be interesting to know whether there is any connection between the position of the air space and the fact noted by Murray (1826) that the temperature of the albumen at the blunt end is higher than that of the other.

All birds' eggs which I have examined have the air space at or near the blunt end of the shell. This holds for all pigeons' eggs in which a more pointed end is distinguishable. Some pigeons' eggs, as Coste noted, have equally rounded ends. In such eggs we find the head of the embryo turned away when we hold the air space end to our left. The practical importance of this for the present study is obvious (see p. 341).

C. *Shell Pigment.*—See discussion on p. 333.

D. *The Chalazæ.*—v. Baer says (1837, p. 18), "ueberhpaut ist nichts im Ei so wechselnd als die Hagelschnüre." Previous to 1850 this variability gave occasion for much study and controversy concerning chalazæ. The earliest workers were quite in the dark as to their nature. Aristotle was the first to refer to them and he thought one of them identical with the stalk of the

ovarian ovum as has been said. The echoes of this opinion did not die out until quite recently; witness the belief that the inner shell membrane is identical with the theca folliculi interna of the oöcyte, an idea advanced by Pander (1818), Meckel v. Hemsbach (1851), Mayer (1865), Nathusius (1885) and others. Needless to say none of these workers had any accurate knowledge of the oviducal egg of any bird. The theory was however not conclusively refuted until 1884 when Tarchanoff succeeded in inserting an amber bead into the oviduct of a laying hen and twenty-four hours later found it surrounded by albumen, shell membrane and shell (cf. Curtis, 1914).

Aldrovanus considered the chalazæ to be the sperm of the cock and Fabricius ab Aquapedente (1625) had a similar idea for he supposed that they were added at the time of fertilization. He considered one of them as the first rudiment of the embryo and drew excellent figures of chalazæ which have a ludicrous resemblance to chick embryos of the fourth day. Wolff in 1764 confesses to having made the same error in his own juvenile studies of the hen's egg. Harvey was the first to give a correct account of the nature and functions of the chalazæ. However he says that the chalaza at the blunt end of the egg is greater and longer than the other, differing in this from all subsequent observers.

A word should be said at this point concerning the various layers of albumen found in the bird's egg. Immediately surrounding the yolk and intimately fused with the vitelline membrane is the chalaziferous albumen which is continuous with the chalazæ. Around this is a layer of fluid albumen which makes possible the independent rotation of the yolk. Next follows the dense albumen which at laying is attached to the shell membrane at either end of the shell (ligamentum albuminis) so that the chalazæ are surrounded by the dense albumen. The space between this layer and the shell membrane is filled for the most part by a very fluid albumen which runs out as soon as the shell is opened. The inner thin albumen is difficult to identify in the laid egg of the hen (cf. Curtis and Pearl, 1912, p. 102) but Doctor Blount tells that this albumen is very obvious in the "uterine" egg of the pigeon. This is obviously an egg in which the origin of the chalazæ can be most readily studied.

The development of the chalazæ was first described by Dutrochet (1818–19). His work was confirmed and extended by Purkinje (1825), v. Baer (1828 and 37) and Berthold (1829) among others. The albumen which at first surrounds the newly escaped ovum forms a thin but dense layer over its surface. This albumen is probably secreted according to Curtis (1915) in the infundibulum and neck of the duct. It coagulates into the membrana chalazifera Dutrocheti and is shortly so firmly united with the vitelline membrane that they cannot be separated. "The tendency, says v. Baer, of albumens in contact with solids to coagulate is well known." The chalaziferous membrane is directly continuous with strands of albumen which were secreted into the lumen of the oviduct above and below the ovum. These strands, attached as they are to the ovum, become the chalazæ. According to Coste (1847, p. 291) the ovum is not free to orient itself with reference to gravity until a fluid layer forms between the chalaziferous albumen and the inner dense albumen probably during the period of shell formation in the "uterus." This might happen while the thin solution of albumen is passing through the developing shell as described by Pearl and Curtis (1912). While in the uterus the egg is constantly being turned on its long axis and the ovum is constantly tending to orient itself with reference to gravity since the animal pole is specifically lighter than the vegetal. Consequently the chalazal strands attached to the yolk become twisted in opposite directions and as a result there is a partial coagulation of the chalazal albumen resulting in the "hailstones."

The stage at which the yolk is first free to rotate independently of the surrounding thick albumen and the time when the chalazæ as such become visible have not been satisfactorily determined. The nature of the space, too, between the chalaziferous albumen and the inner albumen which makes the rotation possible is most obscure. The suggestion of Taschenberg (1885), that the twisting of the chalazæ takes place before the egg enters the uterus is highly improbable. Not infrequently in the hen and pigeon at least, there is more chalazal albumen below the ovum than above it as Purkinje first noted (1825) and consequently the cloacal chalaza is larger than the other. This condition may play

a part in the differentiation of the two ends of the shell. I have records of 14 oviducal pigeon eggs which had more albumen cloacally of the ovum but no instance of the converse. Many observers have noted for the hen that the pointed end chalaza may be larger than its mate in the laid egg. Thus: Maitre Jan (1722, p. 16), Leveille (1799, p. 53), Vicq d'Azyr (1805) who says that the chalaza at the pointed end is "ordinairement" greater than the other, as well as Purkinje (1830, p. 18), v. Baer (1828), Berthold (1829) and Coste (1847, p. 293). Occasionally the infundibular chalaza is wanting in the incubated egg or is represented by a small button or cap of denser albumen (cf. Dutrochet (1818), Purkinje, 1830, p. 18). My own observations for these points are tabulated on p. 354. Since any difference in size or distinctness of the chalazæ is practically always in favor of the cloacal (pointed end) chalaza, we may say that a difference in the chalazæ is a frequent differentiation of the principal axis.

E. *Ligamentum Albuminis.*—Tredern's original description of the ligamentum albuminis occasioned much interest and controversy soon after its publication in 1808 but recent generations seem to have forgotten it entirely. It is familiar to all who have opened fresh eggs at home with a view to strict economy. It should be said that usually it cannot be made out in stale or incubated eggs; there is in fact the same reversal from gel to sol phase in the denser albumen which may occasionally be observed in the chalazæ. This tendency is much more marked in the sparrow's than either the hen's or the pigeon's egg so far as my observations go.

F. *The Long Axis of the Ovum.*—There are two opinions as to the shape of he yolk of the bird's egg. One is the common traditional view, namely, that it is a sphere. Thus Harvey, transcribing his discussion of the yolk largely from Aristotle describes its shape as "perfectè rotundus." Likewise Haller (1758) and Vicq d'Azyr (1805) refer to "la forme spherique du jaune." Pander is one of the careful observers who makes a similar statement and even in Coste's beautiful figures (1847), the outline of the yolk is always a circle. Purkinje himself seems to intimate that the yolk in the laid hen's egg is spherical as his collaborator Valentin (1835) did and as most moderns do, to

judge from their diagrams. Other workers seem to have doubted this tradition. Bonnet (1762) says, "La jaune est un grand sac, a peu pres rond" and Home (1822) comments upon the "oval shape of the yelk." The figures in Tredern's paper and in Duval's Atlas (1888) show the long axis. Now far from being spherical the yolk has not only a longest axis perpendicular to the polar axis but it is usually somewhat flattened at the animal pole so that the polar is its shortest axis. von Baer was the first to describe accurately and understand the significance of the shape of the yolk. Two passages are particularly worth quoting. They were written soon after the appearance of the first part of his great "Ueber die Entwickelungsgeschichte der Thiere" in 1828 although they were not published until 1837. Thus on page 14 he says: "Ihre (der Dotterkugel) Form ist nicht völlig kugelig, sondern ellipsoidisch, *indem ihre längste Axe wie die längste Axe des Eies gerichtet ist.*" Again, page 29, "Da die Dotterkugel im Eierstocke so liegt, dass die Keimschicht fast immer dem Stiele des Kelches zugekehrt ist, da ferner die trichterförmige Mündung des Eileiters den Kelch von der Seite umfasst, so tritt die Dotterkugel in solcher Lage in den Eileiter ein, dass die Keimsicht nicht vorn oder hinten ist, sondern an der Seite. Vorzüglich wird aber diese Stellung dadurch bedingt und mehr gesichert, dass diejenige Axe, die von der Keimsicht durch den Mittelpunkt des Dotters geht, *auffalend kürzer ist* als die senkrecht auf dieser Axe stehende.[1] Letztere wird daher bald in die Längenrichtung des Eileiters gestellt werden, wie auch der Dotter eingetreten seyn mag.

This remarkable description, which may be confirmed by anyone who will make careful measurements, has been almost universally ignored. While v. Baer was the first to see that the ovum orients itself with reference to its long axis in the oviduct—

[1] Hiernach wird es auch verständlicher, warum die Keimschicht, wenn sie nicht in der Nähe des Stieles vom Kelche ist, sich zuweilen in der Narbe zeigt. Sie bleibt nämlich im Kleinsten Kreise des Dotters. In ganz kleinen Eiern von der Grösse eines Hirsekornes habe ich diese längliche Gestalt nicht mit Sicherkeit zu erkennen vermocht. Sollte sie schon da seyn, so könnte man vielleicht sagen, dass das Keimbläschen gegen die nächste Stelle der Oberfläche des Eies sich bewegt und eben deshalb die Centralhöhle, als urpsringlicher Sitz des Keimbläschens, der Keimschicht (einer Wirkung des Keimbläschens) näher liegt, als der entgegengesetzten Seite. (See Bartelmez, 1912, pp. 287 and 295.)

he thought *after* ovulation, it must nevertheless be remembered that practically every objective drawing of an entire ovarian oöcyte shows a well-defined long axis. Pfeil describing a mature oöcyte of the hen in 1823 says ". . . vitello in sacco *scilicet* rotundo, globoso" and Purkinje in 1825 (p. 1) mentioned the 'somewhat oblong' shape of the young oöcytes. He saw also the elongate shape of the mature oöcyte but thought it was due to the pressure of the oviduct (see pp. 9 and 20, 1830 edition).

Allen Thompson (1839) and Balfour (1874) completely confirmed von Baer's description while several workers have described the long axis in ovarian oöcytes of the hen without recognizing its significance. The following may be mentioned: His (1868), Nathusius (1868), Sonnenbrodt (1908) and Riddle (1911).

The observations of Haswell (1887) are particularly significant in this connection. With v. Baer's description in mind he studied the egg of the emu, found the yolk measuring 70 × 75 mm., the long axis coinciding with the principal axis and the relations of the embryo as they are in the hen. He found the yolk ovoid rather than oval in shape, the more pointed end corresponding to the pointed end of the shell. I have occasionally seen the same condition in the pigeon's egg, but in this form attached no particular significance to it, as in some such cases the more pointed end was directed toward the blunt end of the shell.

In the pigeon the yolk is more markedly elliptical than in the hen and since it does not lose its shape through imbibition of albumen during the first two days of incubation as the hen's egg is apt to do, the long axis can hardly escape notice. It averages ten per cent. longer than the other axes of the yolk (see p. 342). It was a matter of no small gratification to find three years after writing it that I had almost duplicated v. Baer's description of the orientation of the hen's egg in my account of the pigeon's egg (1912). In his day the problems of bilaterality had not yet come to the fore and so he did not take the next logical step and point out the significance of the fact that the embryonic axis is definitely related to the ovarian long axis.

In most pigeon's eggs we find the polar axis the shortest; that is, in addition to being shorter than the long axis, it is usually shorter than the axis perpendicular to these two—which I term the transverse axis (Bartelmez, 1912, p. 290 and p. 342

below). Haller made mention of this in 1758 and Féré (1897) has called particular attention to it.

G. *The Oviducal Orientation.*—How does the egg lie in the oviduct? What is the relation of the principal egg axis with its differentiations to the axis of the oviduct? This has always been a matter of interest and it has been very naturally associated with the question as to which end of the egg is laid first. Aristotle says the blunt end is laid first and his whole argument concerning the "principium ovi" seems to assume that this end passes first down the oviduct. Purkinje published the data for the understanding of this matter in 1825 (see especially pages 21 and 22 of the 1830 edition) and he was fully confirmed by von Baer (1828–1837) and Coste (1847), but this was all forgotten and the question was discussed in the seventies and eighties to little purpose. It was finally put to rest by Wickmann in 1895.

It should be said first that all observers who have made extensive studies of oviducal stages in birds agree that the pointed end passes first down the oviduct. For the hen the following may be cited: Purkinje (1825), von Baer (1828), Coste (1847, p. 293), Kütter (1878), Taschenberg (1885), Wickmann (1895) and Patterson (1910). In the pigeon the pointed end when recognizable was always found to be cloacal by Blount (1909) and Patterson (1909), each of whom studied about 150 oviducal eggs, and this has been my own experience. The same conditions have been reported for a hawk by Kütter (1878), the canary and various other birds by Wickmann (1895). It may be said in passing that Meckel von Hemsbach announced in 1851 that it was a mathematical necessity for the *blunt end* to pass *first* down the oviduct! J. A. Ryder (1893) has also worked out the mechanics of shell formation on this basis.

There are however numerous observations which leave no doubt but that the blunt end of the hen's egg is usually laid first as Aristotle said. Nathusius gathered data in 1885 and obtained only a single authentic record of the pointed end having been laid first. Landois (1877), König-Warthausen (1885), Jasse (1886) and Erdmann (1886) all gave evidence from direct observations or from the direction in which the blood streaks of pullet's eggs were rubbed, that the blunt end is laid first. Nathusius (1885) reported a series of observations by Frau A. Ernst

in Caracas upon 48 eggs laid on coal soot. It was clear that in every case the blunt end had been laid first.

It was assumed by these writers that the egg is forced out of the uterus in the act of laying by a simple peristalsis and hence that the blunt end passes first down the oviduct. Wickmann (1895) made direct observations upon the laying of eight hens and found that in most cases the blunt end is laid first; one hen however laid five successive eggs with the pointed end first. If the bird is taken some time before an expected laying the egg is always found with the pointed end cloacal, but, as both Purkinje and v. Baer noted, if she is killed near the time of laying either end may be directed toward the vagina. Purkinje suggested that during "labor" the egg was turned about its long axis until its position became "comfortable." He figured the oviduct of a hen with an egg in the "uterus" (Tab. II., Fig. 19), which shows the pointed end so held in a diverticulum of the duct that it is to the right and below the opening of uterus into vagina. Consequently the constant peristaltic movements of the duct rotate the egg without tending to drive it into the vagina. Wickmann (1895) was the first to analyze the whole process of laying. He demonstrated in a striking manner that in laying the egg is rotated about its shortest axis so that one end or the other lies opposite the opening into the vagina. Usually it is completely rotated so that the blunt end comes to be cloacal, occasionally there are birds in which the rotation is slight and the pointed end remains cloacal. Now a surprising thing happens. The egg is not simply forced out through the vagina as a mammal is born. The uterus still enveloping the egg is prolapsed through the vagina and the egg is laid without having touched the walls of either vagina or cloaca. Which end is laid first then depends upon chance or the peculiarities of the individual oviduct. There is then nothing to militate against the evidence that the end of the egg corresponding to the pointed end of the shell passes first down the oviduct and we may accordingly refer to this end as the *cloacal* end of the egg. Taschenberg (1894) states that certain individuals of *Corvus frugilegus* are apt to lay eggs with the pigment wreath at the pointed end of the shell, instead of the blunt end and that eggs may be found lying

inverted in the "uterus" of this species. In such cases it is probable that the egg is rotated late in its uterine history.

Shell Pigment.—The unequal distribution of the pigment can now be discussed. While the method of distribution has been worked out satisfactorily, the origin of the pigment still remains obscure. As this field is *terra incognita* to most embryologists and ornithologists it is worth a digression. Wicke in 1858 laid the foundation for the understanding of the matter. He discovered that the pigment spots which are various shades of red or green, are closely related to or identical with the blood pigments bilirubin and biliverdin. Leuckart (1853) suggested that they might be derived from blood extravasated from the hyperæmic oviducal vessels. Kütter (1878) made the first direct observations on the subject. He found in the "uterus" of a *Falco tinnunculus* an unpigmented egg, although the eggs of this species are normally deeply pigmented. Scattered over the surface of the oviduct were small red-brown masses presumably passing down, for in other oviducts he found similar masses being incorporated into the outer layers of the shell. He suggested that these pigment masses were secreted in the upper part of the duct. In 1885 Taschenberg confirmed these observations and showed that there are no pigment glands in the oviduct, confirming Nasse's (1862) previous observations. He concluded therefore that the pigment must be derived from the follicle at the time of ovulation. He pointed out for the first time the significance of the greater massing of pigment at the blunt end with reference to the passage of the pigment down the duct and the normal position of the egg in the uterus. It is obvious therefore that the unequal distribution of the pigment clearly marks the principal egg axis and shows that in all cases where there is unequally distributed pigment, the egg is oriented as it is in the hen and pigeon. In 1893 Wickman published a monograph on the pigment of the shell dealing with numerous species, confirming Kütter and independently reaching conclusions similar to those of Taschenberg. He did not however obtain any conclusive evidence for the origin of the blood pigment from the ruptured follicle. This is an opportunity for an exceedingly interesting study involving also the nature of the oxidations of the blood pigments.

The Oviducal Orientation.—We have seen that one end of the principal axis may differ from the other in regard to the shape and pigmentation of the shell, the position of the air space, the ligamentum albumenis, in the size and character of the chalazæ, and the relation of the egg to the oviduct. The vital link in the evidence for the thesis that the principal axis is an expression of an ovarian axis of symmetry was obtained when it was found that the end of the long axis of the yolk which is to pass first down the oviduct is predetermined in the ovary (Bartelmez, 1912, p. 292 ss). In the pigeon at least, the latebra of Purkinje is, like the blastodisc, lighter than the mass of yellow yolk. Purkinje (1830, p. 7) believed the converse for he was much struck by the resemblance of his "fluid-filled cavity" to a plummet and did not make any tests. He did know however that the contents of the latebra have the same histological structure as the white yolk about the blastodisc. His figure (Tab. I., Fig. 16) of a median section of the yolk of a hen's egg shows the center of the latebra nearer the animal pole than the vegetal. This is still more marked in the pigeon's egg (see Bartelmez, 1912, figs. 38 and 39). This specifically lighter latebra is nearer one end of the long axis than the other end and so one end of the ovum gravitates toward the cloaca and is received first into the oviduct as was described in my 1912 paper. It is worth noting that in three clearly objective drawings in the literature the latebra is shown nearer one end of the long axis of the hen's ovum; they are v. Baer's Figs. 1 and 2, Plate III. (1828) and Duval's Fig. 21 (1889). This observation can be made with certainty only on oöcytes during the final growth period or shortly after ovulation. Eggs taken subsequently show more or less diffusion between latebra and surrounding yolk so that accurate measurements are usually impossible (cf. my figures just mentioned). Purkinje made mention of this fact in 1825. It will be remembered in this connection that the yolk increases in weight as it passes down the oviduct, taking up water from the surrounding albumen during its progess (cf. Curtis, 1911).

Since I was able to show that the position of the latebra nearer one end of the long axis is determined by the corresponding position of the germinal vesicle in earlier stages it is possible to trace the principal axis with one end morphologically different

from the other, to the youngest oöcytes in the ovary. These facts have been confirmed by my subsequent observations and the complete demonstration for the pigeon requires only experimental evidence .

H. *The Relation of the Embryo to the Principal Egg Axis* (*Axis Angle*).—Who was the first to describe this remarkable relation? No one has ever tried to find out. Duval (1884) has often been given credit for it, but he was only one of the few who have published concrete data on the subject. These data and the use he made of them are largely responsible for the deeply rooted tradition of today that the embryo in the hen's egg lies at right angles to the principal egg axis. It speaks volumes for the mighty influence which the classic "Atlas d'Embryologie" has had upon all of us. Duval himself gives the credit to Balfour and Kölliker as Kionka also does in 1894, but neither of these appears to have mentioned the matter except incidentally in their text-books. Dareste (1891) and Rabaud (1908) attribute it to v. Baer. v. Baer wrote one of the best and most complete accounts that has ever appeared in the 1828 edition of his epoch-making work. It is difficult to understand how this discussion has come to be so completely forgotten. But v. Baer himself speaks of the relation as a matter of common knowledge at the time. The earliest mention I have succeeded in finding is in Pander's thesis (1817). In the German edition illustrated by D'Alton which immediately followed the original he says (p. 9):

"Sobald die Längenpole des Embryo, *welche nicht dem Längen-sondern dem Querdurchmesser des Eyes entsprechen* gesetzt sind, entsteht zwischen den beiden Falten (des Primitifstreifens) der Länge nach, ein zartes, oben rundlich, unten lanzettförmig breiter Streifen, das Rudiment des Rückenmarkes." The casual nature of the statement leads me to think that he was merely stating an accepted fact which was not particularly significant for the matter in hand. As many workers before the day of physiological salt solution opened the egg at the broad end by way of precaution it may not have been discovered much before this time.

It is possible that Malpighi (1672) noticed the variability between the principal egg axis and the embryonic axis. In all of his figures the embryo is drawn with the head *up* on the page and in most it is parallel to the sides of the page. In certain

young ones however (Tab. I., Figs. 2, 3, 5, 6 and 7 and Tab. II*a*, Fig. 1) the long axis of the embryo is inclined to the vertical. In all but one of these the angle is less than 90°. As it is probable that he drew all the younger blastoderms *in situ* on the yolk, it may be that they had been oriented with reference to the chief shell axis. I did not find any reference to this point in the text.

The relation is best summed up in the words of v. Baer (1828, p. 12):

"Lage des Embryo.—Um die vierzehnte oder fünfzehnte Stunde tritt das erste Rudiment des Embryo auf. Dieses besteht keinesweges in den beiden Primitivfalten Pander's, sondern in einem mittlern Streifen, der etwa 1½ Linie lang ist, und den ich Primitivstreifen nenne. Er ist der Vorläufer der Wirbelsaule und liegt in der Längenachse des durchsichtigen Fruchthofes. Die Längenachse des Fruchthofes entspricht aber *nicht* der Längenachse des Eies, sondern der Querachse desselben, und zwar liegt der Kopf des zukünftigen Embryo, der in dem ersten dunklen Streifen schon durch ein etwas dickeres Ende angedeutet wird, nach links, das Schwanzende nach rechts, wenn man das Ei in seiner Längenachse so vor sich stellt dass das stumpfe Ende dem Beobachter zu- und das spitze Ende abgekehrt ist, der Keim aber nach oben liegt. Hiernach ist die linke Seite des Embryo nach dem stumpfen Ende des Eies gerichtet, die rechte nach dem spitzen Ende. *Indessen ist diese Lage nicht immer so bestimmt, dass die Längenachse des Embryo mit der Längenachse des Eies genau einen rechten Winkel bildete*, der Winkel weicht vielmehr so ab, dass die erstere bald auf der einen, bald auf der anderen Seite sich mehr der letztern nähert, so dass, freilich in sehr seltenen Fällen, beide Achsen fast zusammenfallen können, wobei dann der Kopf des Embryo bald dem stumpfen, bald dem spitzen Ende des Eies zugekehrt ist. Nur einmal fand ich den Embryo umgekehrt liegen, so dass sein Kopf in *der* Hälfte des Eies lag, in der das Schwanzende hätte liegen sollen."

The first writer who gave statistics on axis angles is Dalton (1881). He made observations on 100 hen's eggs holding the pointed end of the shell to the right and noting in which octant the head lay; in other words he grouped his observations in 45° classes. I have transposed his data so that they may be directly compared with my own.

0°	1 per cent.
45°	26 per cent.
90°	50 per cent.
135°	8 per cent.
180°	3 per cent.
Inversions	12 per cent.

(see p. 347 below).

Dalton also took cognizance of the relation between the ovum and the embryo for he suggests that the position of the embryo on the yolk may be due to the way in which segmentation takes place in the "cicatricula"—referring to Kölliker's suggestion regarding the eccentricity of cleavage. He then remarks that variation in the relation of the embryo to the shell may be due to the manner in which the yolk is received and transported through the oviduct. He at least, had not failed to read v. Baer.

Duval (1884) who does not seem to have ever seen "Ueber die Entwickelungsgeschichte der Thiere" and who speaks of "la loi énoncée par Balfour," was the first to make practical use of the axis angle. He saw that it afforded a means of orienting stages previous to the appearance of the primitive streak and accordingly made a "petite statistique" upon 166 hen's eggs incubated 39 hours. He observed the angle between embryo and shell and to judge from his diagrams grouped them in the following classes:

90°	124 eggs	76 per cent.
circa 65°	13 "	8 per cent.
circa 110°	26 "	16 per cent.
180°	2 "	1 per cent.
(Inversion) 270°	1 egg	0.6 per cent.

The large number of cases at or very near 90° may be due partly to the personal equation for which there are many loopholes in his method of measurement, partly perhaps to his having obtained his eggs from a small flock of hens.

Féré (1900) published the most extensive data that have appeared on the hen's egg, in connection with his demonstration that such experimental eggs as develop normal embryos have no more variation in the axis angles than the controls do. His results are tabulated as percentages of "deviations." He considers all cases which show a variation of 45° or more from the "norm," as "deviations." Expressed in my terms this means there is a normal variation of 90°, from 45 to 135° which corre-

sponds exactly to what I found to be the greatest variation in the eggs of an individual pigeon (see p. 347). His results may be summarized as follows:

Total control eggs with normal embryos	1,337
Total experimental eggs with normal embryos	1,588
Percentage of normal eggs with deviation of more than 45°	21.39 per cent.
Percentage of experimental eggs with deviation of more than 45°	28.82 per cent.

In 1908 Rabaud published an exceedingly interesting study upon variation of axis angles in the hen's egg. He determined first from a series of 105 eggs taken at random that as much variation might be met with normally as was reported by Féré, Blanc, and Ferret et Weber in their experimental series. This is shown in the following tabulation of his results:

0°	3
10– 40°	2
45°	20
50– 70°	18
90°	9
110–130°	15
135°	14
140–170°	5
180°	0
270° (inverted)	12

Rabaud showed also by a series of observations on over 100 eggs with windows in the shell that in the course of the first five or six days' incubation the axis angle does not change.

For the pigeon Patterson (1909) gives the usual axis angle as 45° (see my 1912 paper, p. 300), whereas Blount (1909) mentions variations such as 60° and 150°, but regards these as "comparatively rare." My own results which showed much greater variability for the pigeon's eggs and indicated less variability for the eggs of a given bird are discussed on page 347 below.

The only other reports on the relation of embryo to egg in birds are those of Haswell (1887) for the emu and Féré (1896) for the duck. The former found all the relations described by v. Baer for the hen's egg. The embryo was "usually at right angles" but "not infrequently oblique though never longitudinal" to the principal egg axis. Féré (1896) compared the axis angles in the duck with the hen and found they agreed except that the duck showed greater variability. In a series of 74 eggs of each kind, 75 per cent. of the duck eggs showed a

"deviation" of 45° or more as compared with 30 per cent. of the hen's eggs.

In a clutch of six sparrows' eggs I found all the relations typical of the pigeon's egg including the long axis of the yolk and the ligamentum albumenis. In two of these the axis angle could be measured and was found to be 45° in one, and about 125° in the other.

III. Axis Angles.

Before considering the relations found in the eggs of the individual birds it is necessary to consider the normal range of variability in the pigeon's egg. The curve, Fig. 2, shows that the variability is great, in fact, one may meet with almost any angle from 0 to 180 degrees. The extremes met with were 3 and 180° both of them in eggs which had abnormal chalazæ and were therefore not included in the curve. The latter is unique as there were no cases found between 135° and 180°. All similar cases in which the absence of well marked long and chalazal axes made it impossible to measure the angle accurately were omitted, thus eliminating about 7 per cent. of the eggs studied. As is indicated in the curve, the maximum variation was 127° in normal eggs; there were two cases at 135 and one at 8°, all three of them perfectly normal typical eggs in other respects.

The curve of axis angle variability which I published previously (p. 303) shows four modes. These seemed to indicate that a flock of birds could be divided into groups laying eggs with similar angles. In plotting the present more extensive data the curve (Fig. 2) has been compressed by making 10 instead of 5 degree classes and it suggests another interpretation. It shows a resemblance to a curve of normal variability with its mode at 70° and were there several thousand instead of a trifle over five hundred cases I believe the resemblance would be much more striking. It suggests in other words that the actual angle between embryo and principal axis is a matter of chance. It should be noted that 85 per cent. of all cases lie between 45 and 90°. This means that in the pigeon egg the right side of the embryo is practically always (98 per cent.) directed toward the pointed end of the shell, but that in addition the head is usually inclined in the same direction. Obviously Patterson (1910) was correct as to the essential relation between the pigeon embryo

and the chalazal axis but the axis angle is usually greater than he believed. We cannot be more specific concerning the axis angle in the pigeon than to say that in the great majority of eggs

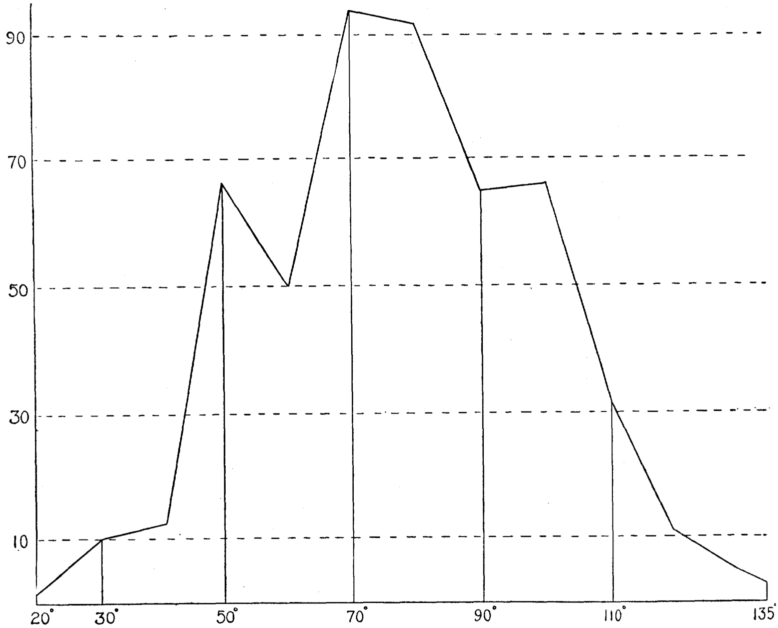


FIG. 2. Curve of variability in axis angles plotted from observations on 506 eggs from 90 different birds. The angles could be measured accurately to 5°. The eggs were grouped in 10° classes and the number of cases in each class plotted as ordinates, the angles as abscissæ. The resemblance to a symmetrical curve of normal variability is apparent.

the head of the embryo lies in the second octant of the (animal) polar hemisphere.

THE AXIS ANGLES OF INDIVIDUAL BIRDS.

My previously published data indicated that the eggs of a given bird show less variation in axis angles than do the eggs of different birds. The following birds were studied for four years, certain of their young mated and their eggs likewise observed. All the birds were the mongrel "homers" of the dealers. The original pairs were given me by Professor Whitman in 1910. They had the freedom of a large attic room where they were free from disturbance, could choose their mates, build their nests and keep comparatively happy throughout the year. At the time I thought there might be some relation between sex

and axis angle variation so in view of Professor Whitman's results the layings were not forced (see Riddle, 1914). When the eggs were removed dummies were left in their places for about two weeks until the birds recognized the deceit or the futility of further incubation and made preparations for another laying. From time to time each pair was permitted to hatch and rear young. Under these conditions the average number of clutches per year was 16, a figure which has been considerably lowered by inclusion of the records of the old birds at the end of their active laying period. The spring was the most prolific season, the autumn the least so, as Pearl and Surface (1911) observed for the hen.

The angles were measured as described in 1912. As some pigeon eggs show little difference between the two ends of the shell the position of the air space was always noted; its end of the shell is readily seen when the egg is placed in the salt solution since it floats up higher than the other. The blunt or air space end of the shell was held to the left, the other end removed with forceps and the contents drawn out. Thus any possible confusion between the two chalazæ was avoided. Except during the first year the eggs were left under the birds for two to three and one half days. As pigeons are usually very lax about incubating until the second egg has been laid the embryos varied from the five to the twenty somite stages. During this period the embryonic axis can be accurately observed and there is normally not enough yolk digestion or absorption of albumen to interfere with the identification of the long axis of the yolk.

Pigeon No. 3.—This was an old bird at the outset to judge from her subsequent activity. The first egg was laid March 16, 1910, the last January 14, 1912; ten weeks later she was killed and a normal resting ovary found. During these 22 months she laid 29 clutches without a single abnormal or infertile egg.

TABLE I. (Pigeon No. 3.)

Year.	Normal Eggs.	Inversions.	Only One Egg in Clutch.	Infertile Eggs.	Abnormal Embryo.	Eggs Incubated.	Eggs Lost.	Totals.
I	25	0	0	0	0	10	1	36
II	21	0	1	0	0	2	0	23
Entire period of activity 1 yr. 10 mos.	46	0	1	0	0	12	1	59

All of the twelve eggs that were incubated to hatching produced normal young.

The only irregularity during almost two years was the laying of one clutch with a single egg. This is usually due to the failure of the first or second egg to enter the oviduct at ovulation or to its escape soon after. More rarely only a single ovum matures at a time.

The range of variability (Fig. 3, *3*) is striking as it ranges from 90 to 130 degrees, whereas it is uncommon in the pigeon to find the angle greater than 90 degrees. The actual variability is small, and while this might be attributed to the small number of cases I am inclined to think the small number of extreme variants is to be correllated with the exceptionally well marked long axis and the uniformly normal chalazæ. Clutch 395 may serve as an example of the size relations of the axes of the ovum:

First egg (primitive streak embryo) 21.8 mm. (long axis), 19.5 mm. (transverse axis), 18.0 mm. (polar axis).

Second egg (10 somites) 20.7 mm. (long axis), 17.6 mm. (transverse axis), 18.0 mm. (polar axis).

We have in this case then a clue to the amount of variability which we must expect in eggs of a single bird where the ovum is normal and there are no irregularities in the functioning of the oviduct. That is to say, in this case the variations due to imperfect orientation in the oviduct and errors of observation due to abnormal chalazæ have been practically eliminated.

Pigeon No. 4.—This bird and her mate had recently paired when first observed. The first egg was laid January 4, 1910. After sixteen clutches had been laid during thirteen months the male was killed by a Diener. Two months later no. 4 mated again and continued laying for the next three years. A similar experiment was made purposely with no. 6 in order to see whether any difference could be observed between the eggs laid during the two matings. During the next two years no. 4 laid 29 clutches; and six during her last year; none of these last could have hatched as the table shows. She was killed March 21, 1914, when an abnormal egg was found in the "uterus," although ovary and oviduct appeared normal. The abnormality in the last year may have been due to the sperm although the male was apparantly normal and younger than his mate.

TABLE II. (Pigeon No. 4.)

Year.	Normal Eggs.	Inversions.	Only One Egg in Clutch.	Infertile Eggs.	Abnormal Embryo.	Incubated Eggs.	Eggs Lost.	Totals.
I........	27	0	1	1	2	2	1	32
II.......	15	1	2	2	0	8	2	27
III......	27	1	4	1	3	3	0	29
IV	0	0	1	4	1	0	0	6
4 years ..	69	2	8	8	6	13	3	94

This record doubtless includes the bird's entire period of activity. The falling off during the second year was due to the period between matings (the active months of February and March) and the hatching of four clutches later in the spring. As it was she laid 51 clutches in the course of four years; 12 per cent. of the eggs were abnormal but most of these latter were laid during the last year.

The curve of variability (Fig. 3, *4*), has a single mode when 10° classes are used but with five degree classes there is one mode at 70° another at 85°. There is a total variation of 53° from 52 to 105°. 72 per cent. of the eggs measured fall between 65 and 85°. Of the twelve eggs which diverged most widely from the mean 9 showed irregularities in the chalazæ, the others were quite typical. One egg which showed an angle of 35°, twenty degrees lower than any other egg of this bird, was not included in the data plotted, as the long axis was obliterated by yolk digestion. Another egg which showed no well defined long axis had an angle near the other extreme of variation, namely 95°. The long axis was distinct in all the other eggs of no. 4, as may be seen from two examples:

Egg 399 (11 somites) 21 mm. (long axis), 19.0 mm. (transverse axis), 19.0 mm. (polar axis).

Egg 479 (primitive streak) 22.0 mm., 19.4 mm. (transverse axis), 18.0 mm. (polar axis).

Two curves plotted from the eggs laid during the first and second matings are almost identical and so this case affords no evidence that the male has any effect upon the relation of embryo to principal egg axis.

The records of the remaining three pairs are more complete as they were the offspring of the above pairs. Their relationships are as follows:

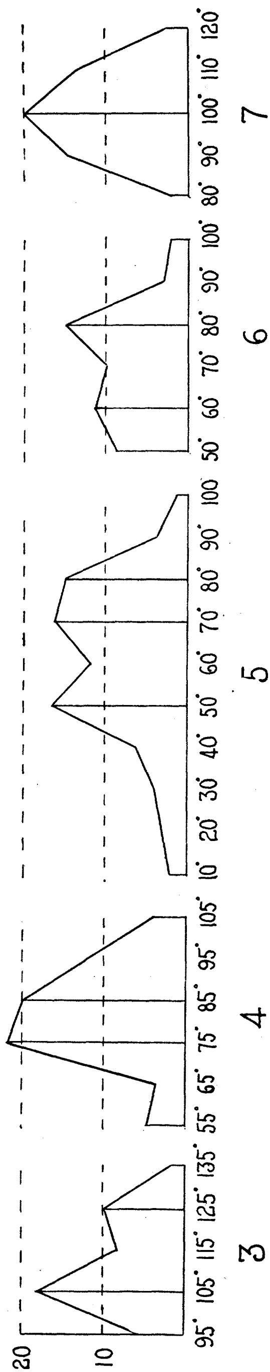
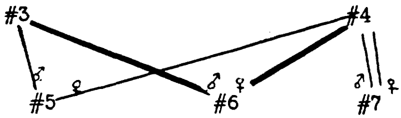


FIG. 3. Curves of variability in the axis angles of pigeons nos. 3, 4, 5, 6 and 7. The curves of nos. 3, 4 and 7 are similar to the general curve (Fig. 2) while nos. 5 and 6 show an exceptional tendency to vary. In every case the individual variability is much less than that of the whole group.



Both no. 5 and her mate were hatched in December, 1910, and mated 4½ months later. The first egg was laid May 5, 1911, the last October 3, 1914, and every egg she laid was studied. Unfortunately her career was cut short by the development of adhesions of the infundibulum which eventually closed the ostium tubæ, and at autopsy the body cavity was found full of yolk. This gradually developing abnormality of the oviduct is to be correlated no doubt with the exceptionally wide range of variability found in her eggs. There were more inversions too (see p. 350) than in any other case.

The table shows that no. 5 was the most prolific of the group, not one of her eggs was infertile and less than 4 per cent. deviated from the normal. The double-yolked egg belongs to Class I. of M. R. Curtis (1915) as both yolks had all membranes in common. It is worth noting that as usual in such eggs the double yolk was oriented with reference to its longest axis while the individual long axes had been obliterated by the fusion. One embryo cut the chalazal axis at 5°, the other at 130°. This is

TABLE III. (Pigeon No. 5.)

Year.	Normal Eggs.	Inver-sions.	Only One Egg in Clutch.	Infertile Eggs.	Abnormal Eggs.	Eggs Incu-bated.	Eggs Lost.	Totals.
I.	32	0	2	0	1	5	5	42
II.	29	2	0	0	1 (double-yolked egg)	4	0	32
III.	19	3	1	0	2	3	1	21
Last five months.	7	1	1	0	0	0	0	7
3 yrs. 5 mos. . .	87	6	4	0	4	12	6	102

further evidence that extremes of variation in the axis angles are due to the failure of an ovum to orient itself in the oviduct with reference to its long axis. The nine extreme variants of this bird showed irregularities in the development of the chalazæ or other abnormalities. The long axis was well marked in all but three eggs. Clutch 611 is typical;

First egg (7 somites)21.3 mm. (long axis) 19.5 mm. (transverse axis), 19.0 mm. (polar axis).

Second egg (4 somites) 21.0 mm. (long axis) 19.8 mm. (transverse axis), 18.0 mm. (polar axis).

Although only three of this bird's eggs had abnormal embryos and all twelve eggs that were incubated hatched, yet her eggs showed the greatest variability (Fig. 3, *5*), a fact which, as has been said, is to be correlated with the abnormal oviduct. One extreme observed was 5°, this was a very abnormal egg as the chalazæ were not attached to the yolk at all but to the outside of the dense albumen. The greatest variation was 84° among the normal eggs.

Pigeon No. 6.—This bird was hatched January 30, 1911, but did not mate until eight months later. The first egg was laid October 29, 1911. Since there were 7 infertile eggs in the next 13 clutches, the male was killed and five months later she mated again with the bird indicated on page 343. In the following two years and two months 24 clutches were laid. The observations had to be discontinued before she ceased to lay, but as she was four years old at the time and had begun to lay numerous abnormal and infertile eggs this record represents practically all her reproductive activity. The high percentage of infertile eggs during the first year was undoubtedly due to her first mate; nevertheless her layings were very irregular and could never be predicted. The curve (Fig. 3, *6*) shows that the axis angles varied

TABLE IV. (Pigeon No. 6.)

Year.	Normal Eggs.	Inversions.	But One Egg in Clutch.	Infertile Eggs.	Abnormal Eggs.	Incubated Eggs.	Eggs Lost.	Totals.
I.	22	0	0	7	0	0	1	30
II.	12	0	1	2	0	8	0	19
III.	21	0	2	4	3 (one was yolkless)	1	2	30
3 years 5 mos.	53	0	3	13	3	9	3	79

in a typical manner, the extremes in normal eggs being 43° and 98°. The most extreme variations were here clearly due to imperfect orientation in the oviduct. Thus two eggs which had no long axis showed angles of 105° and 180° respectively. Most of her eggs had a well-defined long axis; *e. g.*, no. 497, 21.9 mm. (long axis), 18.0 mm. (transverse axis), 17.5 mm. (polar axis). There was no difference to be found in the angles of the eggs laid during the first and second matings.

Pigeon No. 7.—The egg from which this bird was hatched was laid only five days after the last egg of the previous clutch. As it is very rare for a pigeon to lay a second clutch in less than eight days after a first, it seemed possible that the egg might have been laid in no. 4's nest by another bird. However all the active females in the flock had laid within seven days of this date so it seems unnecessary to speculate on the possibility of the parent birds having failed to guard their nest long enough for another bird to lay in it. No. 7 was hatched April 20, 1912, her mate the middle of the following August and they mated in November. The first egg was laid December 2, 1912. The 34 clutches laid during the following two and a quarter years when the work was stopped do not nearly represent the whole active period of this bird.

TABLE V. (Pigeon No. 7.)

Year.	Normal Eggs.	Inversions.	But One Egg in Clutch.	Infertile Eggs.	Abnormal Eggs.	Incubated Eggs.	Eggs Lost.	Totals for Year.
I.	20	0	0	1	1	2	2	26
II.	26	0	0	1	1 (double-yolked egg)	5	1	32
Last three months.	10	0	0	0	1	0	0	10
2 yrs. 3 mos.	56	0	0	2	3	7	3	68

The table shows few peculiarities. The range of variability was low (from 75 to 117°). There were five eggs which had no well-marked long axis; three of them had extreme angles (70°, 117° and 124°), the other two from a single clutch, were 95° and 96°. In this case (clutch 629) the ova measured as follows:

First egg 21.2 mm. (chalazal axis) 21.2 mm. (transverse axis) 19.3 mm. (polar axis).
Second egg 19.2 mm. (chalazal axis) 19.0 mm. (transverse axis) 19.0 mm. (polar axis).

IV. Discussion.

A. *Variability of the Axis Angles.*—The data presented here are the most complete that have been gathered with reference to the axis angles of any bird's egg and they show that while the actual angle may vary considerably, the relation between the head of the embryo and the principal egg axis is very constant. What I have called "inversions" (see p. 349) occur in less than 1 per cent. of the cases. The embryo may lie in almost any position compatible with the general relation between the two axes. If we omit eggs that were not normal as to embryo or chalazæ (circ. 10 per cent. of all), and include only those which could be measured accurately, the extreme axis angles are 8 and 135°. If we compare this maximum with the maximum found for any single bird, namely, 127° as compared with 86°, it is obvious that a given bird's eggs will show less variability than the eggs from a flock. The difference between the two is not fully appreciated until we realize that the maximum variability in the other four birds of the group studied was 40, 42, 53 and 55° respectively; the average maximum variability then for a single bird's eggs is about two thirds of the total variability observed. We may predict that a given pigeon's eggs will show the embryo lying in a quadrant the center of which is usually between 45 and 95° according to the particular bird. In other words the embryonic axis will cut the principal egg axis at an angle which may vary 25° on either side of a mean. The variation we find in an individual bird's eggs makes it clear that the axis angle is no mathematically constant one like the angles of a crystal. There are various mechanical factors operating during ovarian and oviducal stages which undoubtedly contribute to the variability of the axis angle. Thus it is easy to see how

during the growth of the oöcyte the original long axis might be changed more or less, particularly during the final rapid growth period. The oöcyte is free to rotate within its follicle at this time and if it cannot hang down into the body cavity so that attached and animal poles coincide exactly, the long axis of the follicle may not coincide with the original long axis of the ovum. At this time the embryonic axis is already established and so the original relation between long and embryonic axes may be changed (cf. Bartelmez, 1912, p. 288).

The fact that all the most extreme variations were found in eggs with irregular chalazæ or poorly defined long axes makes it certain that difficulties of orientation in the oviduct at ovulation are important factors in axis angle variability. Further, if the chalazæ are not normally attached it is impossible to make accurate measurements. Eleven out of sixteen eggs which had no long axis or very irregular chalazæ were extreme variants for the bird that laid them. It is of course obvious that these factors may partly neutralize each other. This will not explain all the variations observed. We must assume a certain amount of spontaneous variability in the eggs of an ovary.

The first compilations seemed to show that there are always two modes in the curve of individual variation. These appear in Fig. 3, *3*, *5* and *6*, and the same grouping about two modes can also be seen in the case of the eggs of no. 4. It was natural to expect that this might be correlated with the heterozygous nature of the bird's egg (see Riddle, 1912, 1914). That is to say, the larger, female-producing, eggs might vary about one mean, the smaller, male-producing, eggs about the other. The data from the 58 young hatched in the course of the study gave no evidence that the first egg was more frequently male-producing and Dr. Riddle tells me that this condition is found only in pure races of pigeons. Since also the size and weight of incubated yolks varies greatly from the absorption of albumen this possibility could not be tested out.

It is however a matter of great practical importance to see what the chances are that the first and second eggs will have about the same angles. In collecting early stages we usually determine the stage of the second egg from the time of laying of

the first and so the first is available for a measurement of the axis angle. The following table shows the results.

Normal Range of Variability.	Percentage of Clutches in which Both Eggs have the Same Axis Angle.
No. 3, 40° (from 90 to 130°)	45 per cent.
No. 4, 53° (from 52 to 105°)	46 per cent.
No. 5, 84° (from 8 to 92°)	30 per cent.
No. 6, 55° (from 43 to 98°)	45 per cent.
No. 7, 42° (from 75 to 117°)	56 per cent.

The average of these is 43 per cent. of coincidence, but if the very irregular no. 5 be omitted it is 58 per cent.

It should be said further that there is no evidence that the eggs of a mother and daughter tend to resemble each other as to axis angles nor that there is any noticeable difference in the eggs of a bird during a first and second mating or during the early or later parts of her laying period.

We may say in conclusion that two sets of factors contribute toward extreme variation; irregularities in the laying down of the yolk during the final rapid growth period and irregularities in the functioning of the oviduct during the passage of the ovum. Aside from this we must expect normal spontaneous variation to the extent of at least 20 degrees on either side of a mean. This estimate of 40° as the probable maximum spontaneous variation is based upon the records of no. 3 the eggs of which showed no signs of ovarian or oviducal irregularities. Accordingly, if we wish to determine the embryonic axis at a stage when it is not clearly differentiated morphologically, we can distinguish the cephalic from the caudal end of blastodisc or blastoderm in 99 out of 100 cases. The axis angle however, can only be approximated. If the utmost possible accuracy is desired it is necessary to first measure a series of clutches, approximate the mode, eliminate such birds as show great variability, and use this mean angle. It is not likely to be more than 20° wrong in any given egg. Nevertheless, the possibility of even greater variation must be considered in the interpretation of the results.

B. *Inversions.*—By inversions I mean those instances in which the head of the embryo is directed *toward* the observer when the egg is held in the usual manner (Figs. 1 and 4). Von Baer, (1828,

p. 12) as he was the first to discuss axis angles was the first to record an inversion. He observed only one and suggested that it might have resulted from the cracking of the shell before incubation. It is evident from this that he did not appreciate the fundamental nature of the relation between embryo and principal egg axis. Subsequent workers, perhaps on this hint of v. Baer's, have presented evidence that the axis angle may be changed by experimental means. Thus Blanc (1892), Féré (1897 and later), Ferret et Weber (1904) and others have reported not only an increase in the number of extreme variations but also an increase in the number of inversions after poisoning the embryo or after interference with the secondary envelopes. While it is possible that an injured embryo may grow irregularly and so change the original direction of its axis (Féré, 1900) it is highly improbable that it could become inverted. Most of these workers have not adequately determined the normal range of variability of their material. As has been said, the variability may in certain cases be much greater in the hen than in the pigeon. Professor A. P. Mathews tells me that as a student he planned similar experiments but abandoned them when he realized the extent of the normal variation in the hen's egg.

It is interesting to note in this connection that the earlier editions of Foster and Balfour's text-book gave the relations here termed "inversions" as the normal ones for the hen. As Balfour wrote this section of the book it may have been based upon his own observations or it may have merely been an accidental error. Dalton (1881) reported 12 hen's eggs inverted out of 100 while Duval (1884) found only one among 166, Rabaud (1908) 12 in 98 and the writer 33 in 100. For the pigeon on the other hand I reported (1912, p. 301), four cases among over 600 observations by Drs. Blount, Patterson and myself. The present study has thrown some new light on the question.

Inversions are very rare in pigeon eggs. I have studied eggs from about 90 pigeons; while many of them came from the five birds mentioned above, between two and twenty have been obtained from each of the others. Among all these only two birds, no. 4 and no. 5 laid inverted eggs. No. 4 laid two among 94, no. 5 six among 102 eggs. In pigeon no. 5 the axis angles was

exceptionally variable. The other three birds laying an aggregate of 204 eggs had no inversions. This gives a clue to the reason for the great difference in the number of cases reported for the hen. We are again dealing with a matter of individual variation. It is manifest that we should exclude from our studies involving axis angles all birds which show a tendency to vary over 25° from a mean.

How are we to interpret the phenomenon when it does occur? Is it due to an inversion of relations in the ovary or is it more or less accidental? It is always possible that a normal ovum may be unable to orient itself with reference to gravity before ovulation owing to pressure from neighboring ova, adhesions of the

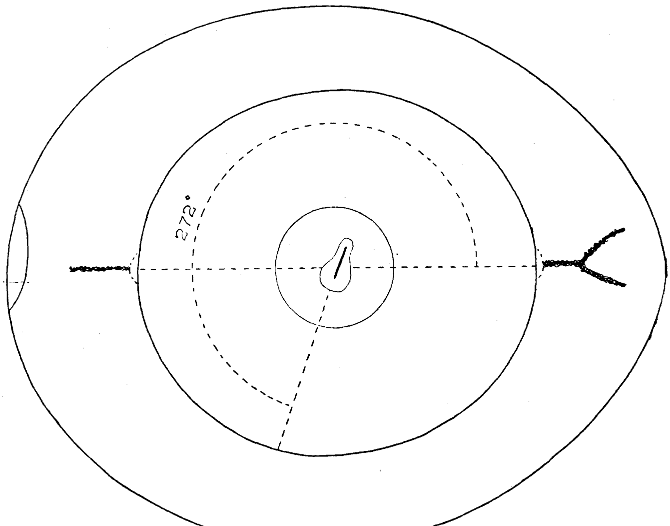


FIG. 4. A diagrammatic polar view of a pigeon's egg showing an "inverted" embryo. This type of inversion can be explained (egg 479′ of no. 4). In this particular case the inversion was very probably due to the fact that the end of the long axis of the yolk which should have entered the oviduct first, entered last.

oviduct, etc., and so the end of the long axis which should have entered first enters last. It may also have fallen into the cœlom at first and have subsequently entered the duct inverted. Since we have complete records of no. 4 and no. 5, the only birds I ever had which laid inverted eggs there is a basis for something more than speculation. When I opened egg 479′ of no. 4 and

found the relations shown in Fig. 4 it was clear that if the egg were turned about the *polar axis* through 180° we would have the usual axis angle for this bird, as 75 per cent. of her eggs had angles between 65 and 85°. Now if the normal orientation of the oöcyte had been accidentally inverted the latebra should be found nearer the cloacal chalaza than the other (*vide* p. 334 *supra*). It was poached, cut through the long and polar axes and the latebra was found 1.3 mm. nearer the cloacal end of the long axis (toward the pointed end of shell) the reverse of the usual condition. It is always possible that the egg was not inverted until after it had entered the "uterus" but before the shell had been laid down (cf. Taschenberg, 1894, p. 308), but I am inclined to think that had this happened the yolk would have been normally related to the air space. The occasional birds' eggs as those of *Corvus frugilegus* (Taschenberg, 1894) which have the pigment wreath at the pointed end of the shell instead of the blunt end should be examined with this point in mind (vide, p. 332 *supra*). We may say then, with some degree of certainty, that in the present case the ovum was inverted at or near the time of ovulation.

The other inverted eggs were studied in the same way but the latebra could not be accurately measured since its boundaries usually become hazy even before laying (see p. 334). However this simple explanation will not suffice for any of the other inverted eggs I examined. A consideration of Fig. 4 will show that it can only hold when the head of the embryo lies in the third quadrant, *i. e.*, between 180 and 270°. The axis angles of the other seven inversions lies between 285 and 325° with the head therefore in the fourth quadrant. If, in these cases, we are to get the relations of embryo and long axis characteristic of the bird we must turn the ovum through 180° about the *long axis* and view the embryo from the vegetal pole as if the yolk were transparent. Obviously this means a reversal of polarity. There is a period in oögenesis when this might happen but I do not see how it could be demonstrated. At the beginning of my "period of differentiation" namely, (1912, p. 286), when the nucleus is nearer the center of the oöcyte than at any other time, it is possible especially in oöcytes with the animal pole nearer the

periphery of the ovary, that the yolk might be so laid down that the nucleus would approach the original vegetal instead of the animal pole. Thus inversion would be accomplished. It should be noted also that three of the inverted eggs of no. 5 were the first eggs of three successive clutches and a fourth was laid soon after, all of them dating from the last six months before the ostium was closed by adhesions.

It is clear from these data that inversions are exceedingly rare in pigeons' eggs, and that only certain individuals ever lay such eggs. There are two types: those which are due to an inversion of the long axis of the ovum at ovulation and those which may be explained by assuming an inversion of the polarity of the oöcyte. It might be expected in such a case that one should find *situs inversus viserum* but this was not noted in the one instance in which the embryo was far enough developed to reveal it (cf. Conklin, 1903).

C. *Variations in the Chalazæ.*—The most accurate and convenient method of measuring the axis angle in an egg is to determine the relation between the chalazal and embryonic axes. Patterson (1909) was, I believe, the first to use the chalazæ for this purpose and I found it by far the most satisfactory method. It is important therefore, to see exactly how variable the chalazæ are and especially how frequently the chalazal axis deviates from the long axis of the ovum which determined it as well as other characters of the principal egg axis. A similar series of observations should be made on the hen's egg where there seems to be much more irregularity.

Typically one finds the chalazæ attached to the chalaziferous albumen at the ends of the long axis of the ovum. As the animal pole flattens out more and more during incubation the points of attachment seem to approach the animal pole, still remaining in the greatest circle of the elliptical ovum. It is rare to find this condition in the pigeon during the first two days of incubation. One often finds a cap or button of clear chalaziferous albumen at the point where each chalaza is attached. Usually when the infundibular (*i. e.*, blunt end) chalaza lies free in the albumen or has become invisible this button marks the corresponding end of the chalazal axis. Again there are eggs which show both

chalazæ unattached and in these there is no chalazal axis. The two chalazæ are frequently unequal in size or form, as was said above (p. 327). Thus only the cloacal chalaza may be forked at the end, or it may be merely longer or stouter than the infundibular one. On the other hand, the two may be indistinguishable. The following table shows the absolute and relative frequencies with which these were encountered and it gives also the frequencies of various kinds of abnormal eggs.

Total number of eggs recorded with complete data...	(447)	100 per cent.
Infertile or abnormally developed eggs.............	(39)	9 per cent.
Eggs lacking the long axis........................	(19)	4 per cent.
Eggs lacking the chalazal axis....................	(14)	3 per cent.
Chalazae equal in size and shape..................	(127)	28 per cent.
Cloacal chalaza recorded greater than other.........	(259)	58 per cent.
Infundibular chalaza absent but button of chalaziferous albumen present...........................	(69)	15 per cent.
Girdle of Vicq d'Azyr present.....................	(2)	0.4 per cent.
Double-yolked eggs...............................	(2)	0.4 per cent.

It should be said that of the 19 cases which had no long axis 5 were either infertile or had abnormal embryos. This was true of 3 which lacked the chalazal axis. On the other hand, irregular chalazæ are much more frequent. Patterson (1909) reported 8 per cent. such eggs, including those which lacked one chalaza. If we make a similar category in the present records, including those eggs which have only a button at the infundibular end of the long axis and those with accessory and unattached chalazæ we get 91 cases (20 per cent.). I have records of only two eggs which had both long and chalazal axes abnormal; approximate measurements of the axis angle can be made in 98 per cent. of normal pigeons' eggs and accurate measurements can be made in 88 per cent. of cases. When the long and chalazal axes do not coincide, as occurs in 2 per cent. of the eggs, they may diverge from 4 to 20°, very rarely more. Most of these were otherwise normal and it should be borne in mind that the same is true of most eggs which lack long or chalazal axes. This emphasizes the fact that this relation is an effect, not a cause; it is simply the morphological expression of the presence of certain axes of bilaterality. The embryo may develop normally whether this expression is perfect or not.

We may say then, that in the pigeon's egg the chalazæ vary

considerably, although extreme irregularities are rare (3 per cent.). Since the two chalazæ were indistinguishable in only 28 per cent. of cases we may say that in the majority of eggs more albumen is secreted below the ovum than above it when it first enters the duct and consequently the cloacal (pointed end) chalaza is frequently larger than the other. Unfortunately I have recorded only 14 of the instances when this was found to be the case in early oviducal eggs.

V. General Conclusions.

1. The older literature on the bird's egg has been grossly neglected by recent students of this subject.

2. The bilaterality of this egg finds expression in two axes of bilateral symmetry: the embryonic axis and the principal egg axis which is defined for the most part by characters of the secondary egg envelopes.

3. These axes are definitely related to each other in such a way that the right side of the embryo is nearer one of the ends of the principal axis than the other.

4. This holds for the eggs of all the species that have been examined with this relation in mind: viz., the domestic fowl, the common pigeon, the emu, the duck and perhaps the sparrow.

5. The actual angle between the two axes (*axis angle*) is subject to great variation.

6. Conclusions from observations on the pigeon's egg.

(*a*) The axis angle was found to vary almost 180° but 85 per cent. of the cases fell between 45 and 90°.

(*b*) Eggs obtained from a single bird vary much less, the maximum variation being from 32 to 68 per cent. of that found in eggs from the entire flock.

(*c*) Extreme variations of the axis angle are probably due to imperfect orientation of the ovum in the oviduct, to abnormalities in its action or to irregularities during the final growth period of the oöcyte. Such eggs usually show irregularities either of long axis or chalazæ.

(*d*) The angle in normal eggs of a given bird, while usually restricted to a range of 45° is apparently determined within this range by the laws of chance during oögenesis.

(*e*) In about one half the clutches studied the angles of the first and second eggs agreed closely. This is a matter of great practical importance in the study of ovarian and oviducal eggs.

(*f*) Inversions are due either to the reversal of the long axis of the ovum at about the time of ovulation or they may be due to the reversal of the polarity of the oöcyte early in oögenesis.

(*g*) The long axis could be identified in 96 per cent. of the eggs studied, the chalazal axis in 97 per cent. The two coincided in 98 per cent. of normal eggs.

(*h*) The chalazae were found abnormal in only 3 per cent. of cases but more or less atypical in 20 per cent. of cases.

(*i*) In 58 per cent. of the eggs the cloacal (*i. e.*, pointed end) chalaza was strikingly larger than the other.

I am indebted to the Librarians of the Surgeon General's library and of the Harvard Library for the loan of several of the older works mentioned and to Miss E. Dickenson of the University of Chicago Library for help in tracing many of the earlier references.

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